

Inhibitory Interactions between BK and JC Virus among Kidney Transplant Recipients

Xingxing S. Cheng,* Daniel L. Bohl,* Gregory A. Storch,*[†] Caroline Ryschkewitsch,[‡] Monique Gaudreault-Keener,[§] Eugene O. Major,[‡] Parmjeet Randhawa,^{||} Karen L. Hardinger,[¶] and Daniel C. Brennan*

Departments of *Internal Medicine and [†]Pediatrics, Washington University School of Medicine, St. Louis, Missouri;

[‡]Laboratory of Molecular Medicine and Neuroscience, National Institute of Neurological Disorders and Stroke,

Bethesda, Maryland; [§]Virology Laboratory, St. Louis Children's Hospital, St. Louis, Missouri; ^{||}Divisions of Transplantation

Pathology, Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania; and

[¶]Department of Pharmacy Practice, University of Missouri, Kansas City, Missouri

ABSTRACT

BK and JC polyomaviruses can reactivate after transplantation, causing renal dysfunction and graft loss. The incidence of JC reactivation after renal transplant is not well understood. Here, we characterized JC reactivation using samples collected during the first year after transplantation from 200 kidney recipients. We detected BK and JC viruses in the urine of 35 and 16% of transplant recipients, respectively. The median viral load in the urine was 400 times higher for BK virus than JC virus. The presence of BK viremia made concurrent JC viremia less likely: JC viremia was detected in 22% of non-BK viremic recipients compared with 4% of BK viremic recipients ($P = 0.001$). The co-detection rate was 1.5%, which is less than the expected 5.6% if reactivation of each virus was independent ($P = 0.001$). We did not observe JC viremia, JC nephropathy, or progressive multifocal leukoencephalopathy. The onset of JC viremia was associated with donor, but not recipient, JC-specific antibody in a titer-dependent fashion and inversely associated with donor and recipient BK-specific antibody. Donor and recipient JC seropositivity did not predict BK viremia or viremia. In conclusion, among renal transplant recipients, infection with one polyomavirus inversely associates with infection with the other.

J Am Soc Nephrol 22: 825–831, 2011. doi: 10.1681/ASN.2010080877

The human polyomaviruses, JC and BK, cause asymptomatic childhood infections and then persist in various sites including the uroepithelium.¹ Reactivation in the renal–urinary system manifests as viremia.^{1,2} In healthy Swiss blood donors, viremia rates were 7 and 19% for BK and JC virus, respectively.² After renal transplantation, the incidence of viremia for each virus increases to 58%.^{3–7}

BK is the major etiologic agent of polyomavirus-associated nephritis occurring in up to 10% of renal transplant recipients (RTRs).^{8–10} JC ne-

phropathy, in contrast, is rare and comparatively benign.^{8–10} Most studies report that urinary co-activation of both viruses in non-RTRs is unusual.^{2,7,11–13} However, large prospective studies examining the interaction of JC and BK reactivation and serology in RTRs are lacking. Our study determined the incidence of JC viremia and viremia in prospectively collected urine and blood samples from 200 RTRs initially tested for BKV; 8 were lost to follow-up and excluded.^{14–17} We also explored the interactions of JC

and BK serology on the incidence of JC and BK infection.

JC viremia was detected in 30 (16%) recipients. Twelve were anuric at the time of transplant, and no pretransplant urine sample was available. The first post-transplant urine samples were collected at 6 to 18 days after transplant; six of these first samples already contained detectable JC DNA but no BK DNA. Of the recipients who developed JC viremia, 16 (53%) were viremic within 1 week, 9 (30%) at between week 1 and 1 month, and 5 (17%) at >1 month after transplantation. The median time to onset of JC viremia was 11 days. No recipient developed JC viremia, and no JC-polyomavirus associated nephropathy was detected. The median JC viremia viral load was 6.39 log₁₀ copies/ml (range 3 to 8.83 log₁₀ copies/ml) compared with the median BK viremia viral load of 8.98 log₁₀ copies/ml determined previ-

Received August 24, 2010. Accepted January 10, 2011.

Published online ahead of print. Publication date available at www.jasn.org.

Correspondence: Dr. Daniel C. Brennan, Washington University in St. Louis, Renal Department, 660 S. Euclid Avenue, St. Louis, MO 63110. Phone: 314-362-8351; Fax: 314-362-2713; E-mail: Brennan@wudosis.wustl.edu

Copyright © 2011 by the American Society of Nephrology

ously.¹⁴ The baseline characteristics of patients (age, gender, race, cause of ESRD, type of transplant, cold ischemia time, HLA mismatch, or type of calcineurin inhibitor used) who did or did not develop JC viruria were similar,

except that recipients with transient JC viruria (positive urine at a single time point) were older (62.3 ± 9.8 versus 45.5 ± 13.6 years; $P = 0.003$). Four pairs of recipients received a kidney from the same donor in this study. In

one pair, both developed JC viruria, but only one from each of the three other pairs developed JC viruria ($\kappa = -0.429$, $P = 0.400$). This lack of concordance suggests that JC reactivation occurs in the native kidney on immu-

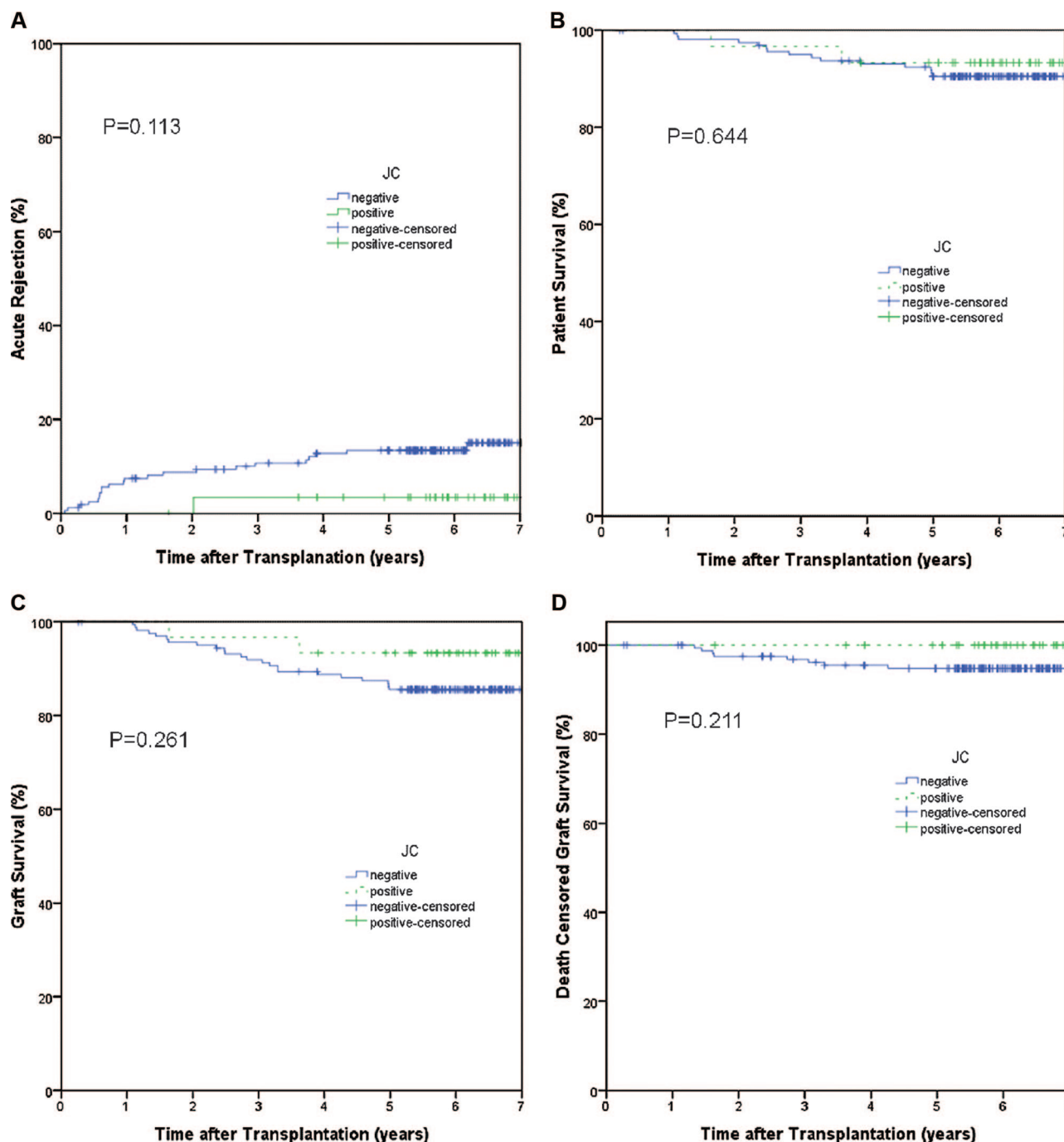


Figure 1. Overall clinical outcome is favorable for those with JC viruria. (A) There was a trend for the incidence of acute rejection to be lower among those with JC viruria in the first year after transplant. (B) Patient survival did not differ between those who had JC viruria in the first year after transplant and those who did not. (C) There was a trend for graft survival to be higher among those who had JC viruria in the first year after transplant. (D) There was a trend for death-censored graft survival to be higher among those who had JC viruria in the first year after transplant.

nosuppression, in contrast to BK, which is most likely donor-derived in the first year after transplantation and reactivates in the donor-allograft kidney.¹⁵ Alternatively, transmission may be relatively inefficient and does not occur in every case when JC viruria is introduced with an allograft kidney. Other factors, such as BK seropositivity or reactivation, may also decrease the likelihood of JC reactivation, as described below.

The clinical outcome of JC viruric RTRs was favorable up to 5 years after transplant. Neither donor type (deceased or living) nor immunosuppressive regimen affected the development of JC viruria. In the first year, acute rejection occurred in 0 of 30 (0%) JC viruric *versus* 11 of 162 (7%) non-JC viruric patients. By 5 years, 2 (7%) JC viruric and 23 (14%) non-JC viruric patients ($P = 0.113$, $P = 0.05$ excluding noncompliant patients) developed acute rejection (Figure 1A). In contrast, there was no change in the acute rejection rate based on BK viruria status, with and without stratification by JC viruria status. JC viruria status did not predict patient survival (Figure 1B). Graft survival ($P = 0.261$) and death-censored graft survival ($P = 0.211$) were not decreased in JC viruric patients (Figure 1, C and D). Serum creatinine did not differ between those with and without JC viruria.

We then looked at JC serology in relationship to JC viruria. Forty-one of the 72 donors (57%) and 93 of the 143 recipients (65%) from whom specimens were available tested positive for JC antibodies. Neither recipient JC serostatus nor JC antibody titer was associated with the development of or time to JC viruria. In contrast, positive donor JC antibody serostatus was associated with the development of JC viruria in the recipient ($P = 0.025$; Figure 2A), and this likelihood increased in a titer-dependent fashion ($P = 0.01$; Figure 2B). JC viruria in recipients of kidneys from JC-seropositive donors also occurred earlier, with a median onset of 13 *versus* 90 days ($P = 0.029$).

BK and JC viruses seemed to have inhibitory interactions. Of the 1133 urine samples tested, 121 (10.7%) contained

detectable JC DNA. JC viruria was detected in 22% of non-BK viruric recipients compared with 4% of BK viruric recipients ($P = 0.001$). JC DNA was detected in 113 (13%) of BK-negative urine samples compared with 8 (3%) of BK-positive urine samples ($P < 0.0001$; Table 1).

Interestingly, the onset of JC viruria

was associated with both donor and recipient BK seronegativity ($P = 0.002$ and $P = 0.05$ in Figure 3, A and B, respectively). The likelihood of developing JC viruria was the lowest in the BK antibody D+/R+ group and highest in the BK antibody D-/R- group ($P = 0.006$; Figure 3C). The likelihood of developing JC viruria also increased in an inverse step-

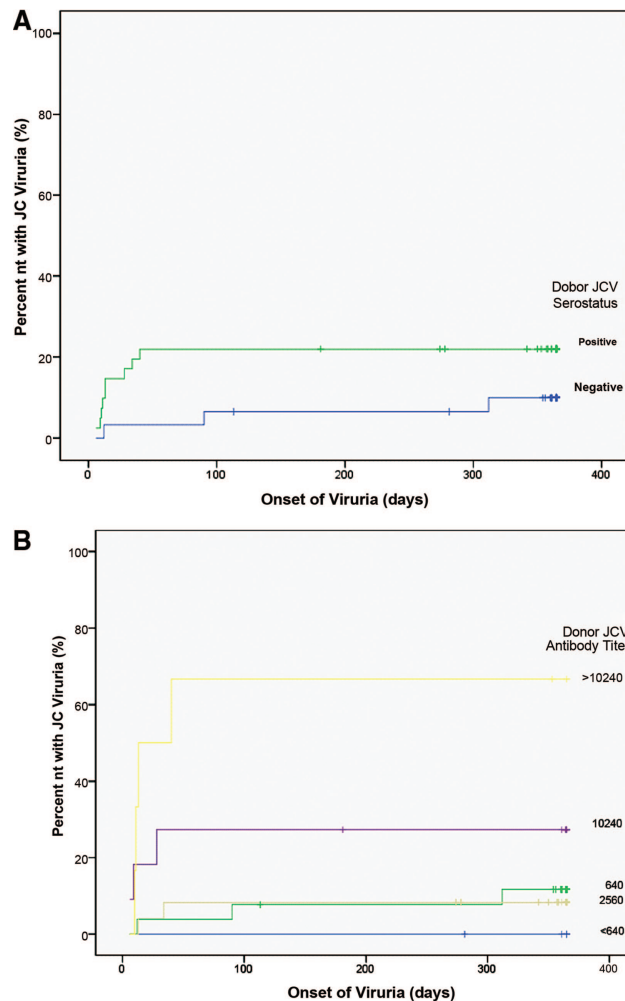


Figure 2. Donor JCV seropositivity strongly associates with development of JC viruria in the recipient. (A) Positive donor JCV serostatus predicts the development of JC viruria in the recipient in the first 60 days after transplant. Donor pretransplant serum samples were available for 72 recipients. At 60 days, the incidences of JC viruria were 3.2 and 22.0% in recipients of kidneys from seronegative and seropositive donors ($P = 0.025$), respectively. Recipients are censored after their last available urine sample (+) or at 365 days. (B) Donor JCV antibody titer predicts the development of JC viruria in the recipient in the first year after transplant in a dose-dependent fashion. Among the 72 donor pretransplant serum samples, the reciprocal JCV Ab titers were ≤ 160 in 5, 640 in 26, 2560 in 24, 10,240 in 11, and $\geq 40,960$ in 6. At 365 days, the incidences of JC viruria by reciprocal antibody titer were 0% for <160 , 11.5% for 640, 8.3% for 2560, 27.3% for 10,240, and 66.7% for $\geq 40,960$ ($P = 0.001$). Recipients are censored after their last available urine sample (+) or at 365 days.

Table 1. Concurrence of BK and JC viruria in recipients in the first year after transplant

Viruria Status	Urine Samples (n = 1133)	<i>P</i> ^a	Recipients (n = 192)	<i>P</i>
BK−/JC− (%)	65.9		49.5	
BK−/JC+ (%)	10.0		14.0	
BK+/JC− (%)	23.4		34.9	
BK+/JC+ (%)	0.7	<0.0001	1.6	0.001

^a*P* values are derived from the χ^2 test.

wise fashion in relation to the donor BK antibody titer ($P = 0.016$; Figure 3D). The median time to onset of JC viruria was not significantly different in recipients of kidneys from BK-seropositive donors compared with seronegative donors (26 versus 13 days; $P = 0.831$). In contrast, no association was observed between donor and recipient JC serostatus and incidence or onset of BK viruria.

It has been hypothesized that JCV and BKV co-activate at low rates in immunosuppressed states. We found a co-activation rate of 1.6%, which is 3.5 times smaller than a predicted rate of 5.6% if the two polyomaviruses reactivated independently. This shows that the reactivation of one polyomavirus is negatively associated with the reactivation of the other in the same patient, a finding not previously reported.

The basis for this negative association between JC and BK is unclear. Active replication by one polyomavirus may interfere with the replication of the other by competing for the same cellular replication machinery or by direct inhibition. Because JC viruria after transplant occurs sooner than BK viruria (median, 11 versus 40 days), early JC reactivation may inhibit subsequent BK reactivation by the above mechanism. Other mechanisms include the immune response responsible for keeping BKV and JCV below pathogenic levels,¹ especially the humoral immune response. Our data suggest that presence of the JC antibody is a surrogate marker for the activity of JC infection in the donor kidney, a relationship similar to that which we observed for BK.¹⁶ Interesting, we also observed an inverse relationship between donor BK antibody titer and recipient JC viruria. We suspect that this is because donor BK antibody titer is associated with recipient BK viruria, and BK viruria seems to have

an inhibitory effect on JC viruria. The inverse relationship between recipient BK antibody titer and JC viruria, however, points to humoral cross-protection. Although these anti-BK antibodies cannot clear well-established BK infections with high copy numbers,^{16,18} they may play a prophylactic role against subsequent JC infection with low copy numbers. Our finding parallels the observation that, in immunocompetent individuals, JC viruria was more prevalent in subjects seropositive for only JC viruria compared with subjects seropositive for both JC viruria and BK viruria.² Future studies are needed to assess adequately the contributions of the innate, humoral, and cellular immune systems in the post-transplantation period.

Clinically, we found a surprising trend for a lower acute rejection rate and improved graft survival among those who had JC viruria that is unexplained by the decreased incidence of BK infection in those with JC viruria. We hypothesize that, because JC viruria is benign by itself and a negative predictor for the more serious BK infection, its presence is a marker of adequate immunosuppression as reflected by a trend toward lower acute rejection and improved graft survival. From a clinical management perspective, these data establish definitively that reduction of immunosuppression in the case of JC viruria (16% incidence in the first year) is not indicated, because it predicts negatively against the more serious BK reactivation and that the long-term outcomes are good, if not more favorable, in JC viruric patients. We cannot say whether JC viremic patients warrant reduction of immunosuppression. However, its absence from our study population of 192 patients confirms previous findings that JC viremia, and by extension JC ne-

phropathy, is not a common clinical problem.^{7,19–21} We have ongoing studies investigating the possible beneficial associations of JC viruria. If it is shown conclusively that JC viruria is associated with lower acute rejection and better graft survival, clinicians may consider incorporating JC viruria monitoring into routine clinical practice.

There are limitations to our study. We used stored samples for analysis. DNA, however, is stable at -80°C . Also, antibody testing was available from only 72 (36%) donors and 143 (72%) recipients. Nonetheless, this is the largest cohort of donor and recipient pairs that have been analyzed for JC and BK antibody and reactivation in renal transplantation to date.

In conclusion, we showed that infection by one polyomavirus is negatively associated with reactivation of the other. We provide evidence that JC reactivation in RTRs is less likely to be donor-derived but, as is the case for BK, recipient JC seropositivity does not protect against JC reactivation. Our data suggest that JC viruria in the absence of JC viremia marks adequate but not overimmunosuppression, potentially leading to a lower acute rejection rate. Monitoring for JC viruria and viremia might provide a way to monitor and adjust immunosuppression.

CONCISE METHODS

Subjects and Samples

Two hundred *de novo* RTRs were followed prospectively for 1 year as part of single center randomized clinical trial.¹⁴ Urine and blood samples initially tested for BK virus were stored at -70°C . The Washington University Human Research Protection Office approved the trial and allowed for additional testing.

Eight patients were lost to follow-up, leaving 192 recipients. Urine and blood samples collected at presentation, 1 week, and 1, 3, 6, 9, and 12 months after transplantation were tested for JC DNA. JC antibody testing was performed on donor and recipient blood samples. Pretransplant

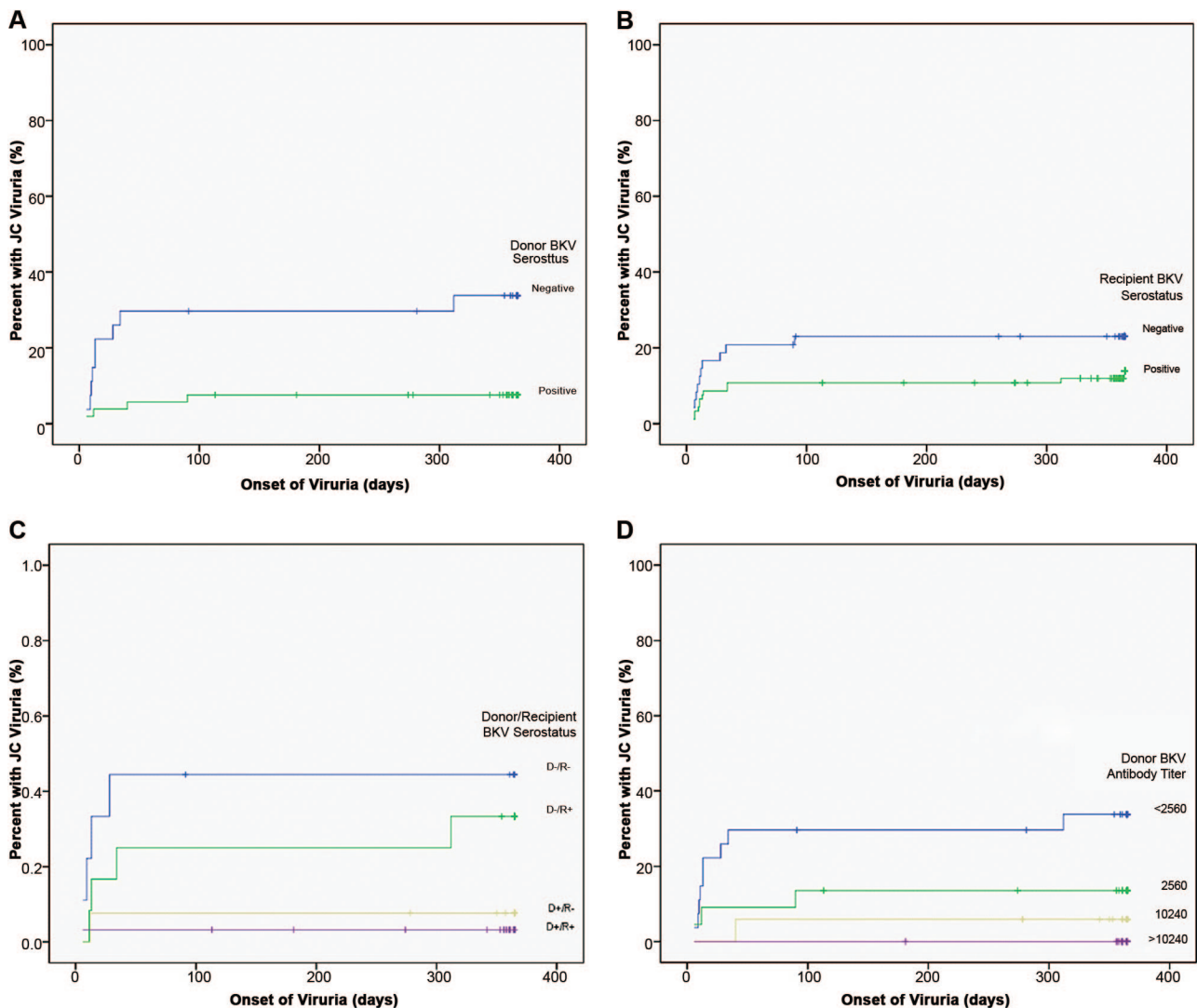


Figure 3. Both donor and recipient BK seropositivity negatively associate with the development of JC viruria in the recipient. (A) Positive donor BKV serostatus is associated with a lower likelihood of the development of JC viruria in the recipient in the first year after transplant. Donor pretransplant serum samples were available for 80 recipients. At 365 days, the incidences of JC viruria were 33.3 and 7.5% in recipients of kidneys from BK seronegative and seropositive donors ($P = 0.002$), respectively. Recipients are censored after their last available urine sample (+) or at 365 days. (B) Positive recipient BKV serostatus is associated with a lower likelihood of the development of JC viruria in the recipient in the first year after transplant. Pretransplant serum samples were available for 141 recipients. At 365 days, the incidences of JC viruria were 22.9 and 10.8% in BK seronegative and seropositive recipients ($P = 0.05$), respectively. Recipients are censored after their last available urine sample (+) or at 365 days. (C) D+/R+ BKV serostatus is associated with a lower likelihood of the development of JC viruria in the recipient in the first year after transplant. Of the 65 donor/recipient pairs, 31 were D+/R+, 13 were D+/R-, 12 were D-/R+, and 9 were D-/R- for BKV antibodies. At 365 days, the incidences of JC viruria by BKV serostatus were 3.2% for D+/R+, 7.7% for D+/R-, 25.0% for D-/R+, and 44.4% for D-/R- ($P = 0.006$). Recipients are censored after their last available urine sample (+) or at 365 days. (D) BKV antibody titer predicts against development of JC viruria in the recipient in a dose-dependent fashion. Among the 80 donor pretransplant serum samples, the reciprocal JCV Ab titers were ≤ 640 in 27, 2560 in 22, 10,240 in 17, and $\geq 40,960$ in 14. At 365 days, the incidences of JC viruria by reciprocal antibody titer were 33.3% for < 640 , 13.6% for 2560, 5.9% for 10,240, and 0% for $\geq 40,960$ ($P = 0.016$). Recipients are censored after their last available urine sample (+) or at 365 days.

samples were available from 72 (36%) donors and 143 (72%) recipients and tested for JC antibody and BK antibody as described previously.¹⁵

JC PCR Assays

A JC-specific quantitative real-time PCR assay was established using the LightCycler System version 1.0 (Roche Diagnostics, In-

dianapolis, IN). Primers JC-F CTATCCA-CACAAGTGGG and JC-R AAAGTTGCT-CATCAGCC were used to amplify a 205-bp region of the gene encoding the JC viruria

large T-antigen. Nucleic acid extraction from plasma and urine samples was performed using QIAamp spin columns (QIAGEN, Valencia, CA) as described previously.¹⁴ The PCR cycling conditions were 95°C for 8 minutes and then 50 cycles of 95°C for 10 seconds, 54°C for 10 seconds, and 72°C for 8 seconds, followed by cooling at 30°C for 30 seconds. Probes JC probe 1FL ACAGGGGCAATGCACTG-FL and JC probe 1RD LC Red640-GGATTAGTG-GCACAGTTAGGCC (TIB Molbiol, Adelphi, NJ) were used to detect the JC viruria PCR product. The positive control was a plasmid containing the JC viruV genome (Advanced Biotechnologies, Columbia MD). The JC viruria assay was performed using 2 μ l nucleic acid extract from plasma or urine and regularly detected a control containing 1000 plasmid copies/ml. The assay was specific for JC viruria and quantified at Washington University. The detection of 100 copies of JC DNA was not inhibited by the presence of 10^7 copies of BK DNA. Five different JC concentrations (1×10^6 , 1×10^4 , 1×10^2 , and 1×10^1 , and 1) were used to generate a standard curve, and quantitative viral titers were calculated by LightCycler software version 1.0. Infections were divided into “sustained” and “transient,” with sustained defined by the detection of JC DNA in at least two consecutive samples spanning ≥ 3 weeks.

BK Antibody and JC Antibody Enzyme Immunoassay

BK- and JC-specific IgG antibodies were measured using virus-like particles (VLPs) made by expressing BK or JC VP1 proteins in baculovirus vectors in insect cells and then preparing the VLPs for use in the ELISA as described previously.^{22,23} Starting at a 1:40 dilution, samples were diluted in plate-wells in serial fourfold increments. A positive threshold was set at a spectrophotometer absorbance of 0.05 greater than that of appropriate serum controls. Samples with antibody titers $\geq 1:2560$ were considered to be seropositive to eliminate concerns for cross-reactivity with other polyomaviruses. Controls included VLPs for BK, JC, or SV40 as appropriate.

Statistical Analysis

Results of continuous data are reported as medians followed by maximum and mini-

mum values. ANOVA was used for comparisons of means between groups. Fisher's exact test was used for comparisons of categorical data. Survival analysis and the log-rank test were used to compare the prevalence of viruria over time.

ACKNOWLEDGMENTS

This work was supported in part by NIH Grants 2 K24-02886 (D.C.B.) and DK 079333 (D.C.B.). These data were presented in part at the National Kidney Foundation 2009 Spring Clinical meeting in Nashville, TN, as Abstract 39.

DISCLOSURES

None.

REFERENCES

- Randhawa P, Brennan DC: BK virus infection in transplant recipients: An overview and update. *Am J Transplant* 6: 2000–2005, 2006
- Egli A, Infanti L, Dumoulin A, Buser A, Samaridis J, Stebler C, Gosert R, Hirsch HH: Prevalence of polyomavirus BK and JC infection and replication in 400 healthy blood donors. *J Infect Dis* 199: 837–846, 2009
- Hogan TF, Borden EC, McBain JA, Padgett BL, Walker DL: Human polyomavirus infections with JC virus and BK virus in renal transplant patients. *Ann Intern Med* 92: 373–378, 1980
- Gardner SD, MacKenzie EF, Smith C, Porter AA: Prospective study of the human polyomaviruses BK and JC and cytomegalovirus in renal transplant recipients. *J Clin Pathol* 37: 578–586, 1984
- Arthur RR, Dagostin S, Shah KV: Detection of BK virus and JC virus in urine and brain tissue by the polymerase chain reaction. *J Clin Microbiol* 27: 1174–1179, 1989
- Boubenider S, Hiesse C, Marchand S, Hafi A, Kriaa F, Charpentier B: Post-transplantation polyomavirus infections. *J Nephrol* 12: 24–29, 1999
- Helantera I, Ortiz F, Auvinen E, Raisanen-Sokolowski A, Lappalainen M, Lautenschlager I, Koskinen P: Polyomavirus BK and JC infections in well matched Finnish kidney transplant recipients. *Transpl Int* 22: 688–693, 2009
- Hirsch HH, Knowles W, Dickenmann M, Passweg J, Klimkait T, Mihatsch MJ, Steiger J: Prospective study of polyomavirus type BK replication and nephropathy in renal transplant recipients. *N Engl J Med* 347: 488–496, 2002
- Ramos E, Drachenberg CB, Portocarrero M, Wali R, Klassen DK, Fink JC, Farney A, Hirsch H, Papadimitriou JC, Cangro CB, Weir MR, Bartlett ST: BK virus nephropathy diagnosis and treatment: Experience at the University of Maryland Renal Transplant Program. *Clin Transpl* 16: 143–153, 2002
- Bohl DL, Brennan DC: BK virus nephropathy and kidney transplantation. *Clin J Am Soc Nephrol* 2[Suppl 1]: S36–S46, 2007
- Kitamura T, Aso Y, Kuniyoshi N, Hara K, Yogo Y: High incidence of urinary JC virus excretion in nonimmunosuppressed older patients. *J Infect Dis* 161: 1128–1133, 1990
- Markowitz RB, Thompson HC, Mueller JF, Cohen JA, Dynan WS: Incidence of BK virus and JC virus viruria in human immunodeficiency virus-infected and -uninfected subjects. *J Infect Dis* 167: 13–20, 1993
- Azzi A, Cesaro S, Laszlo D, Zakrzewska K, Ciappi S, De Santis R, Fanci R, Pesavento G, Calore E, Bosi A: Human polyomavirus BK (BKV) load and haemorrhagic cystitis in bone marrow transplantation patients. *J Clin Virol* 14: 79–86, 1999
- Brennan DC, Agha I, Bohl DL, Schnitzler MA, Hardinger KL, Lockwood M, Torrence S, Schuessler R, Roby T, Gaudreault-Keener M, Storch GA: Incidence of BK with tacrolimus versus cyclosporine and impact of preemptive immunosuppression reduction. *Am J Transplant* 5: 582–594, 2005
- Bohl DL, Storch GA, Ryschkewitsch C, Gaudreault-Keener M, Schnitzler MA, Major EO, Brennan DC: Donor origin of BK virus in renal transplantation and role of HLA C7 in susceptibility to sustained BK viremia. *Am J Transplant* 5: 2213–2221, 2005
- Bohl DL, Brennan DC, Ryschkewitsch C, Gaudreault-Keener M, Major EO, Storch GA: BK virus antibody titers and intensity of infections after renal transplantation. *J Clin Virol* 43: 184–189, 2008
- Hardinger KL, Koch MJ, Bohl DJ, Storch GA, Brennan DC: BK-virus and the impact of preemptive immunosuppression reduction: 5-year results. *Am J Transplant* 10: 407–415, 2010
- Randhawa P, Bohl D, Brennan D, Ruppert K, Ramaswami B, Storch G, March J, Shapiro R, Viscidi R: Longitudinal analysis of levels of immunoglobulins against BK virus capsid proteins in kidney transplant recipients. *Clin Vaccine Immunol* 15: 1564–1571, 2008
- Randhawa P, Uhrmacher J, Pascule W, Vats A, Shapiro R, Eghtesad B, Weck K: A comparative study of BK and JC virus infections in organ transplant recipients. *J Med Virol* 77: 238–243, 2005
- Yogo Y, Kitamura T, Sugimoto C, Hara K, Iida T, Taguchi F, Tajima A, Kawabe K, Aso Y: Sequence rearrangement in JC virus DNAs molecularly cloned from immunosup-

- pressed renal transplant patients. *J Virol* 65: 2422–2428, 1991
21. Drachenberg CB, Hirsch HH, Papadimitriou JC, Gosert R, Wali RK, Munivenkatappa R, Nogueira J, Cangro CB, Haririan A, Mendley S, Ramos E: Polyomavirus BK versus JC replication and nephropathy in renal transplant recipients: A prospective evaluation. *Transplantation* 84: 323–330, 2007
22. Hamilton RS, Gravell M, Major EO: Comparison of antibody titers determined by hemagglutination inhibition and enzyme immunoassay for JC virus and BK virus. *J Clin Microbiol* 38: 105–109, 2000
23. Viscidi RP, Rollison DE, Viscidi E, Clayman B, Rubalcaba E, Daniel R, Major EO, Shah KV: Serological cross-reactivities between antibodies to simian virus 40, BK virus, and JC virus assessed by virus-like-particle-based enzyme immunoassays. *Clin Diagn Lab Immunol* 10: 278–285, 2003